



Yang-Chang YU
Application No. 102005,221

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IN THE TITLE:

Changed
in PALM

Please amend the title as follows:

-- CYTOTOXIC ANNONACEOUS ACETOGENINS FROM *ANNONA MURICATA* --

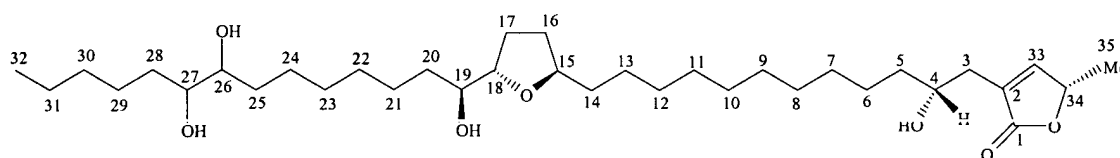
IN THE CLAIMS:

A.E.

Please amend claim 1 as follows:

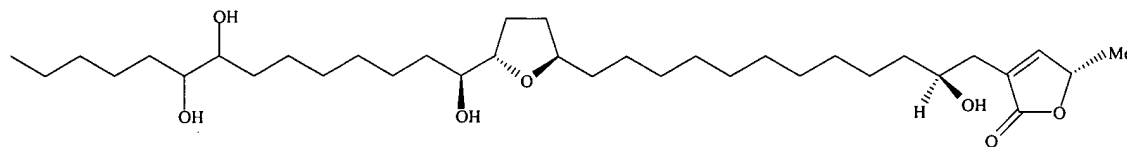
1. (Amended) Isolated and purified Annonaceous acetogenin compounds having the structures of a-g, wherein

a. muricin A has the formula of:



BS
said muricin A having an α, β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a threo conformation, two methylene groups of the mono-THF ring corresponding to a trans conformation, two hydroxyl groups at C-26 and C-27 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration;

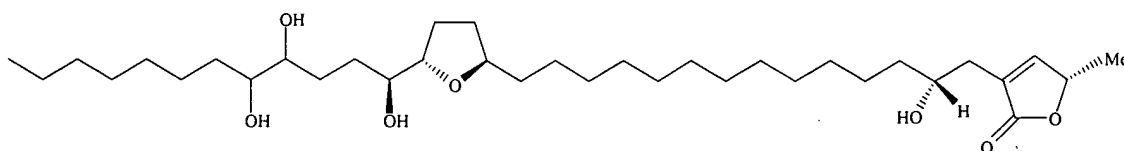
b. muricin B has the formula of:



said muricin B having an α, β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a trans/threo conformation, two methylene groups of the mono-THF ring corresponding to a trans

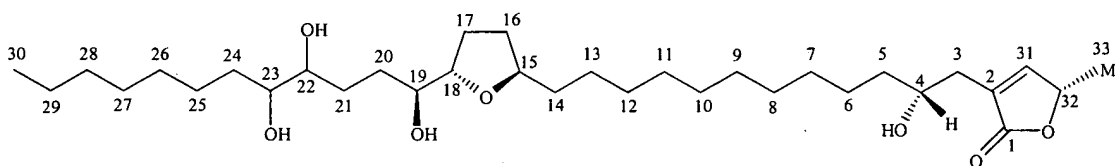
conformation, two hydroxyl groups at C-26 and C-27 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration;

c. muricin C has the formula of:



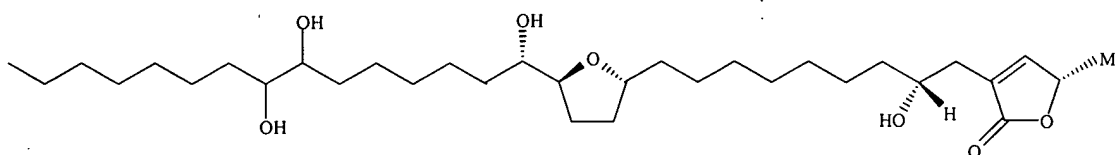
said muricin C having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-17 and C-20 with one flanking hydroxyl in a trans/threo or threo/trans conformation, two hydroxyl groups at C-24 and C-25 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration;

d. muricin D has the formula of:



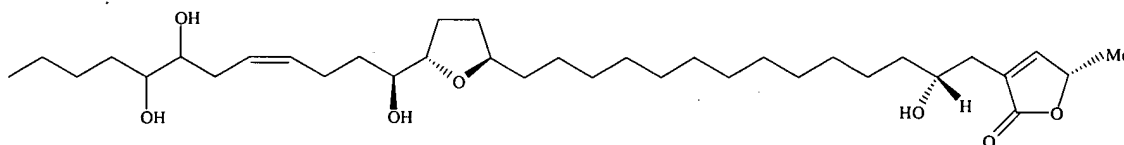
said muricin D having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4 position, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based;

e. muricin E has the formula of:



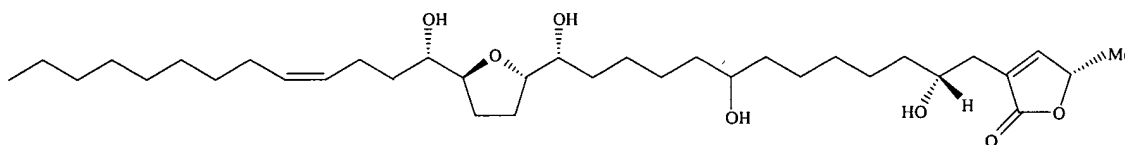
said muricin E having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4 position, a mono-THF ring placed between C-12 and C-15 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based;

f. muricin F has the formula of:



said muricin F having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-17 and C-20 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-27 and C-28 as vicinal diol assigned as threo based, and a double bond determined at C-24/C-25; and

g. muricin G has the formula of:



said muricin G having an α , β -unsaturated γ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-16 and C-19 with one flanking hydroxyl in a threo/trans/threo conformation, one hydroxyl groups formed at C-10, a double bond determined at C-23/C-24, and the stereochemistry at C-34 on the γ -lactone fragment performed in (S)-configuration.

Please amend claim 2 as follows:

2. (Amended) A method for isolating and purifying the Annonaceous acetogenins

compounds according to claim 1 comprising:

extracting muricins from *Annona muricata* seeds with MeOH to obtain a MeOH extract
at room temperature;

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evaporating and partitioning the MeOH extract in a CHCl₃ and aqueous mixture,
whereby said Annonaceous acetogenins compounds are in the CHCl₃ layer of the CHCl₃ and
aqueous mixture; and

further separating the Annonaceous acetogenins compounds of said CHCl₃ layer by
column chromatography.

✓
Please cancel claims 3-4.

✓
Please amend claim 5 as follows:

5. (Amended) An anti-tumor composition comprising an effective amount of at least one
of the Annonaceous acetogenins compounds according to claim 1.

B²
(Please amend claim 6 as follows:)

6. (Amended) The Annonaceous acetogenins compounds as claimed in claim 1, wherein
the Annonaceous acetogenins compounds are used for treatment of patients having a tumor.

✓
Please amend claim 9 as follows:

B³
9. (Amended) A method for treating hepatoma cancer comprising administering to a patient afflicted with hepatoma cancer an effective amount of a pharmaceutical composition comprising at least one Annonaceous acetogenins compounds according to claim 1 and a pharmaceutically acceptable salt and ester in combination with pharmaceutically acceptable carrier, auxiliary or excipient.

Please add new claims 10-18 as follows:

New Claim 10. The isolated and purified Annonaceous acetogenins compounds according to claim 1, wherein said compound is isolated from *Annona muricata*.

New Claim 11. The isolated and purified Annonaceous acetogenins compounds according to claim 10, wherein said compound is isolated from seeds of *Annona muricata*.

B⁴
New Claim 12. The method according to claim 2, wherein said column chromatography is an Si gel column.

New Claim 13. The method according to claim 12, wherein said Annonaceous acetogenins compounds are eluted from the Si gel column by a gradient comprising *n*-hexane-CHCl₃ and CHCl₃-MeOH.

New Claim 14. The method according to claim 13, wherein said Annonaceous acetogenins compounds are further purified by a reversed-phase high performance liquid chromatography.

New Claim 15. The method according to claim 13, wherein said CHCl₃ layer is separated into ten fractions by the Si gel column.